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Figure 1

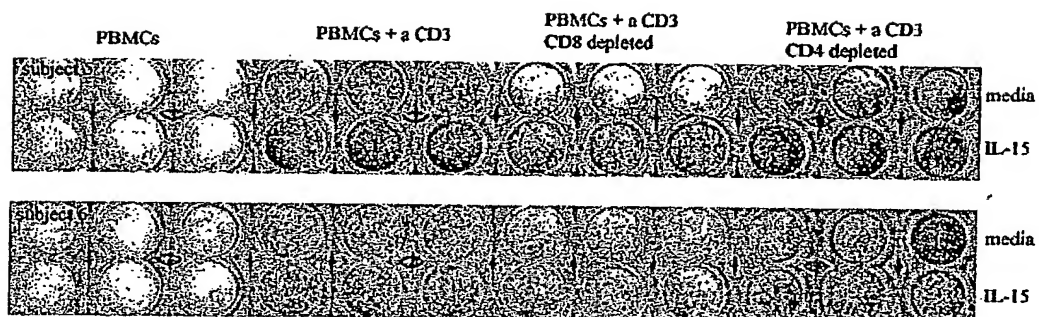


Figure 2

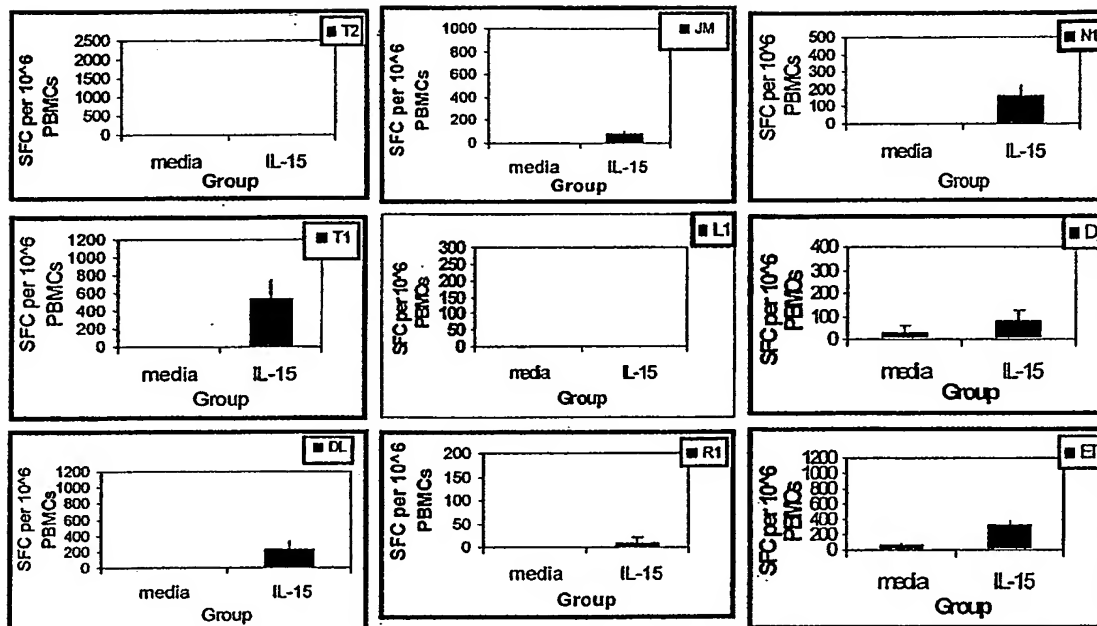


FIGURE 3A

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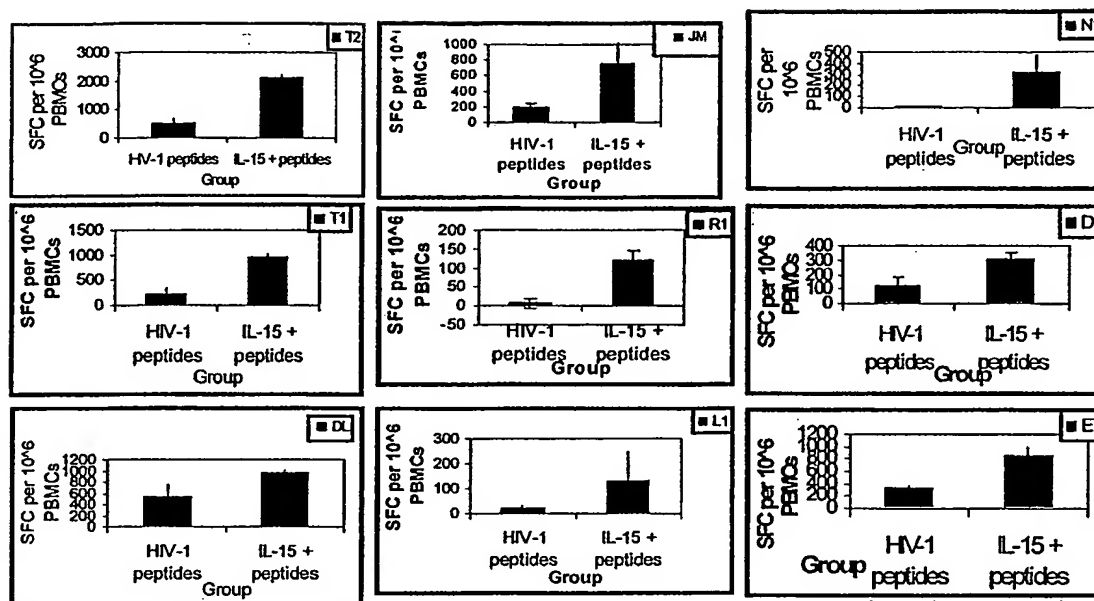


FIGURE 3B

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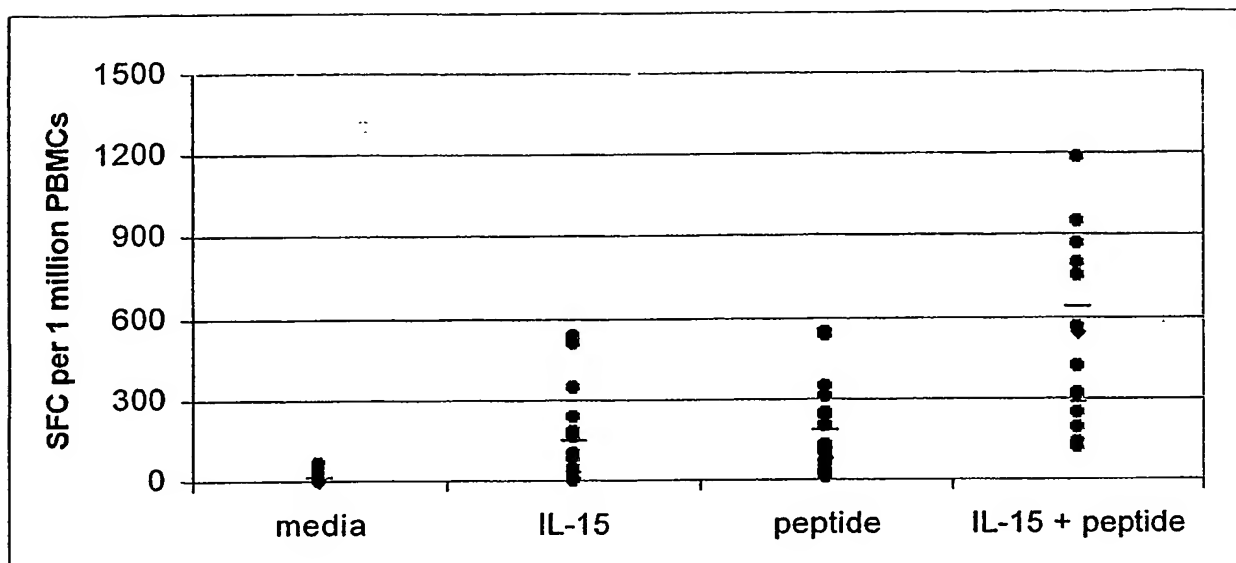


Figure 3C

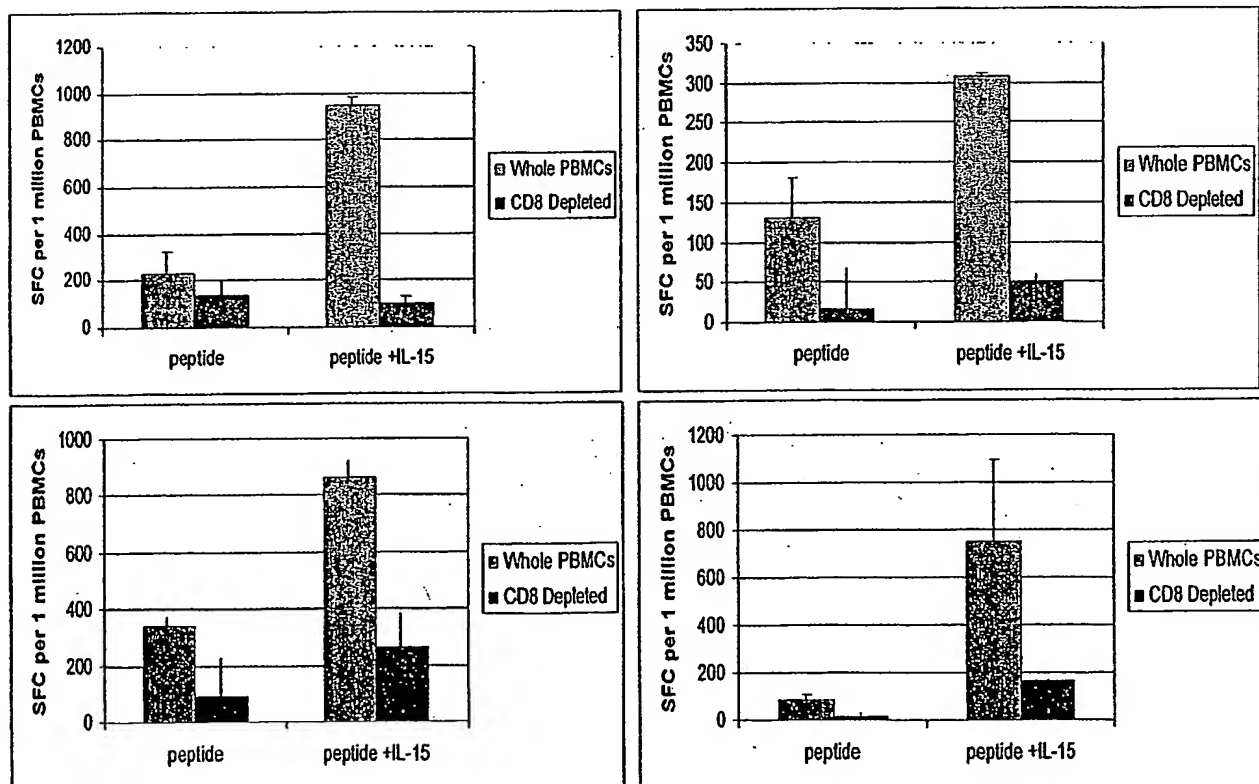


Figure 3D

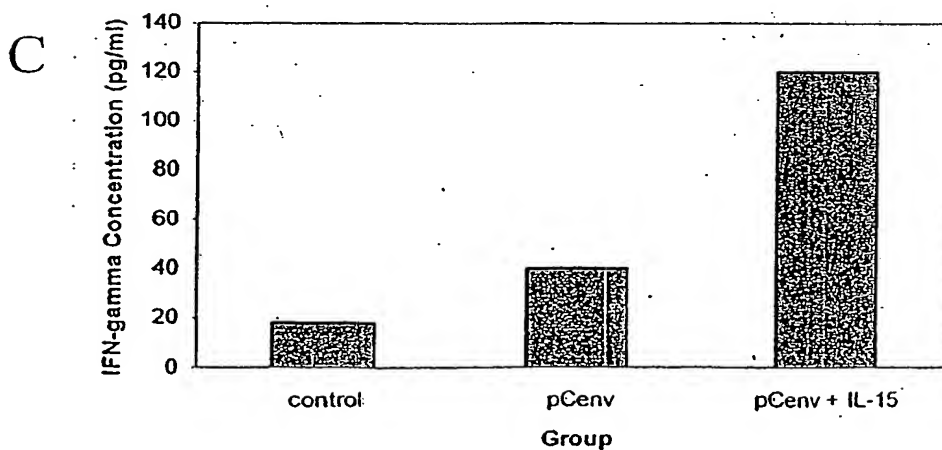
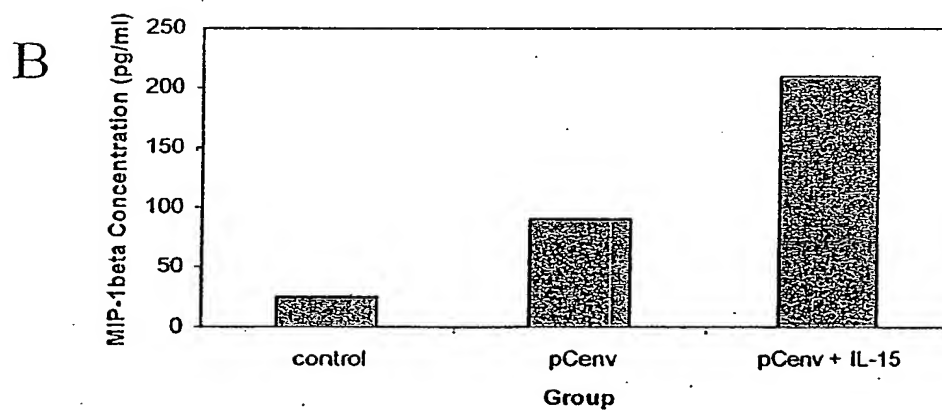
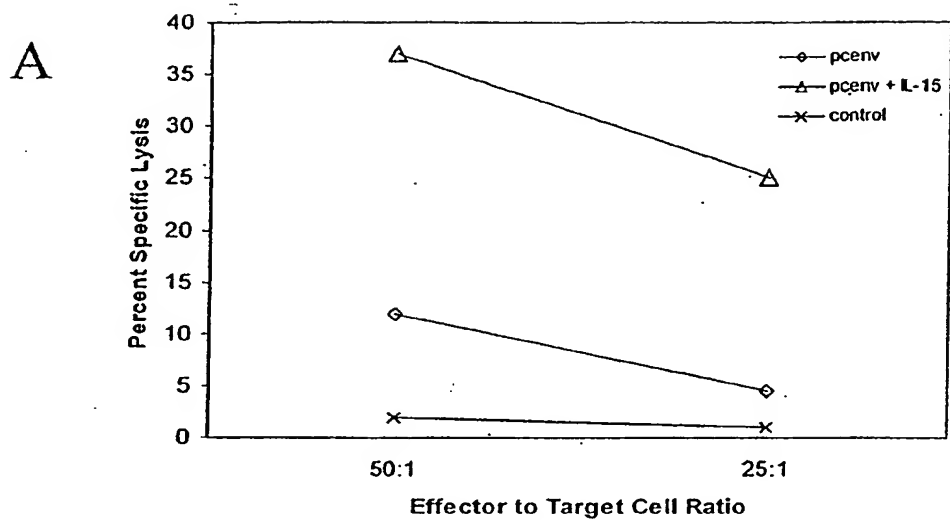


Figure 4

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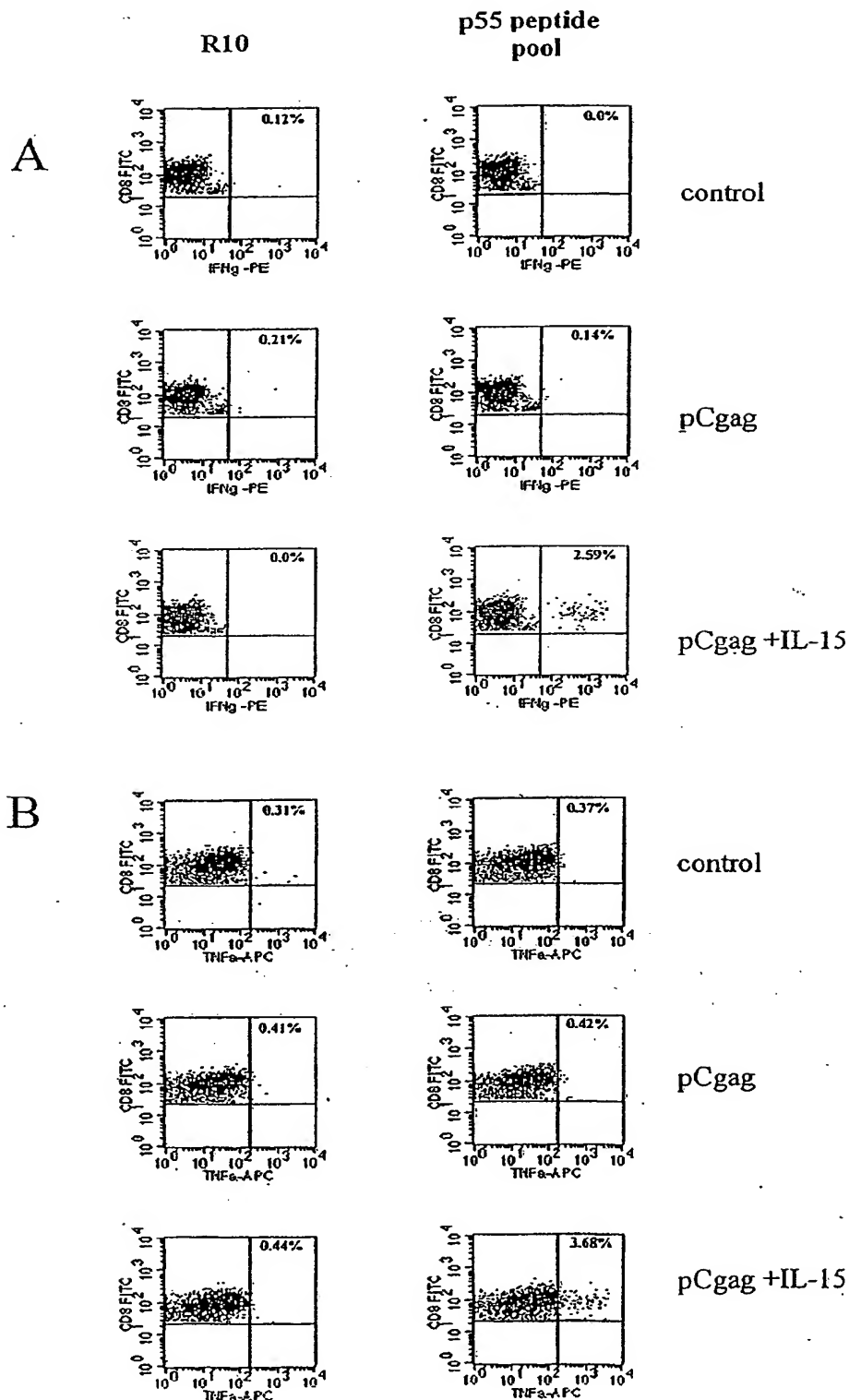


Figure 5

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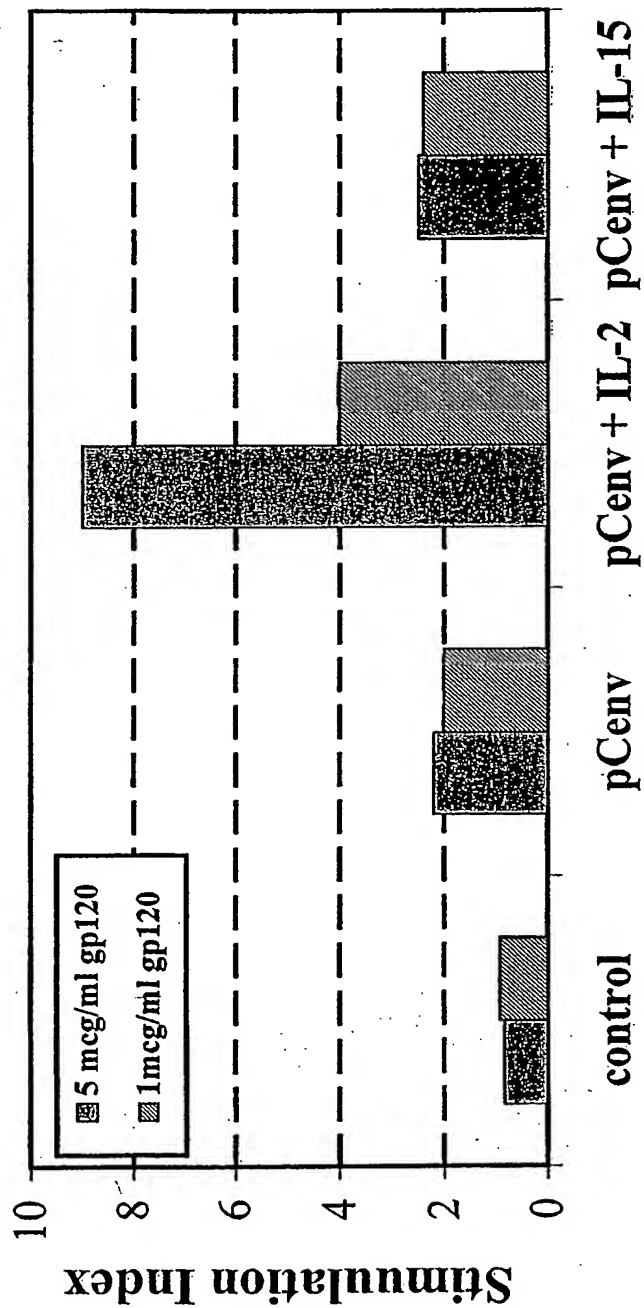


FIGURE 6

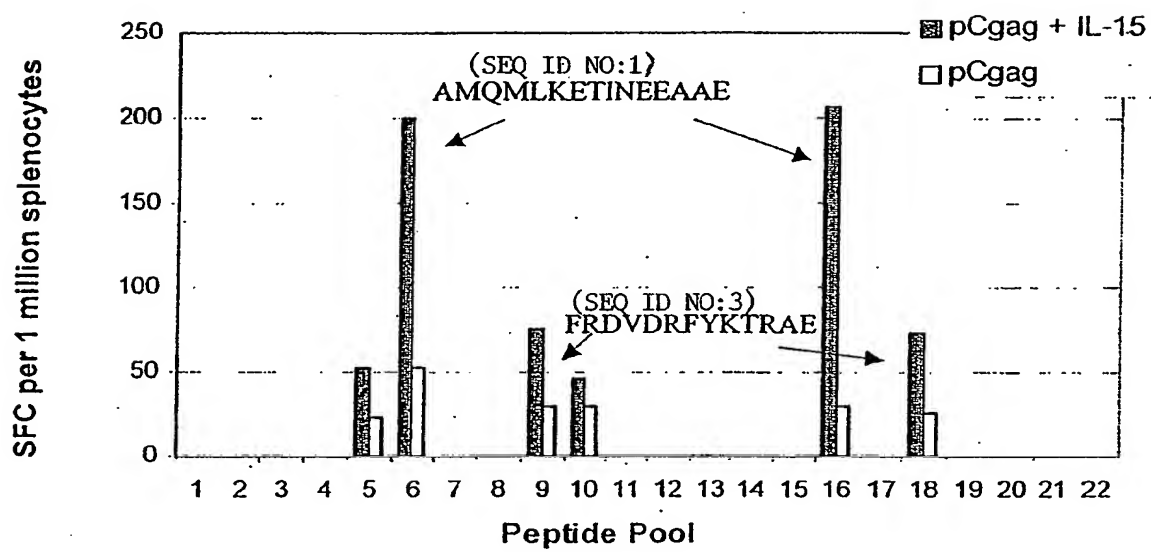


Figure 7

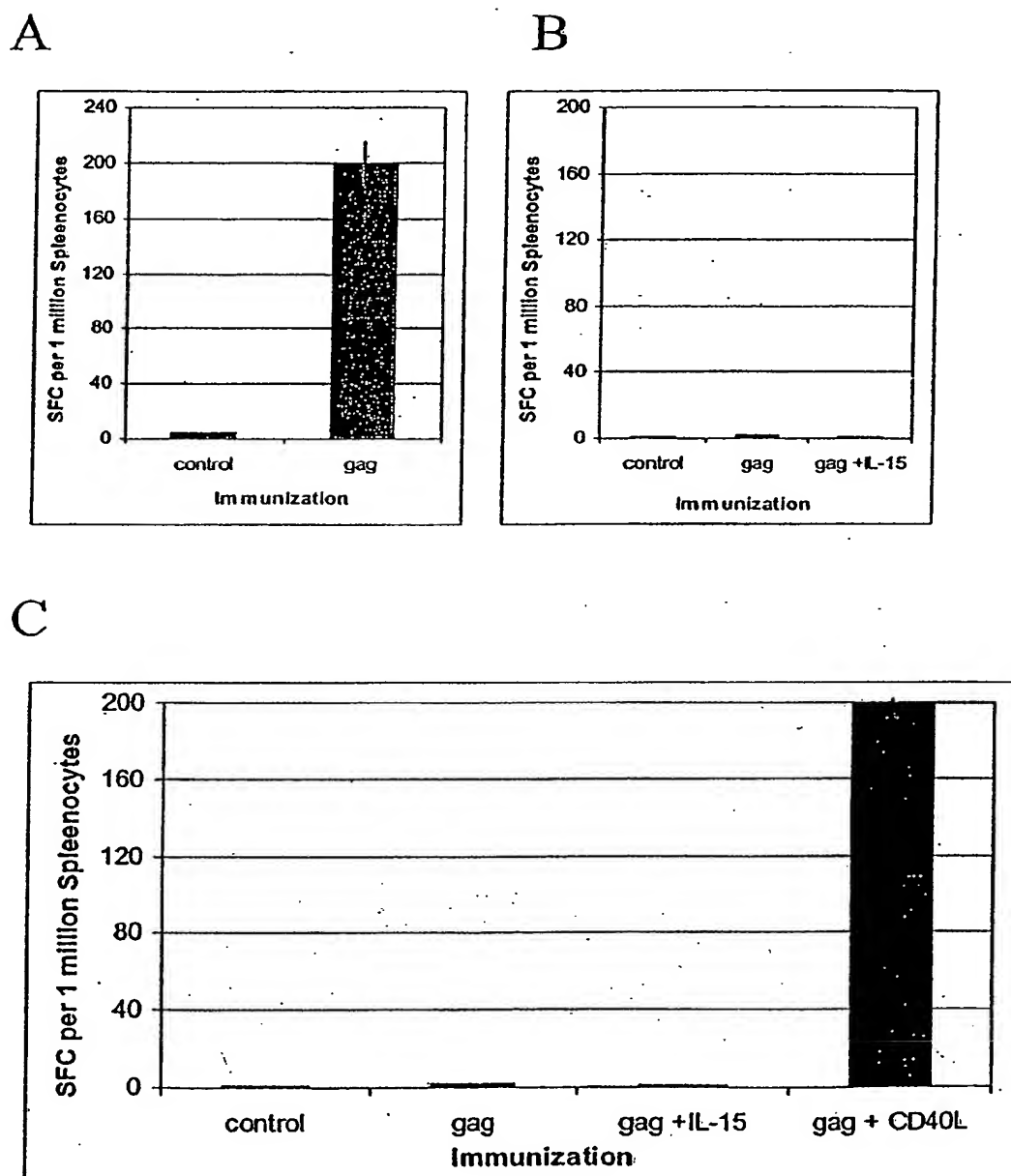


Figure 8

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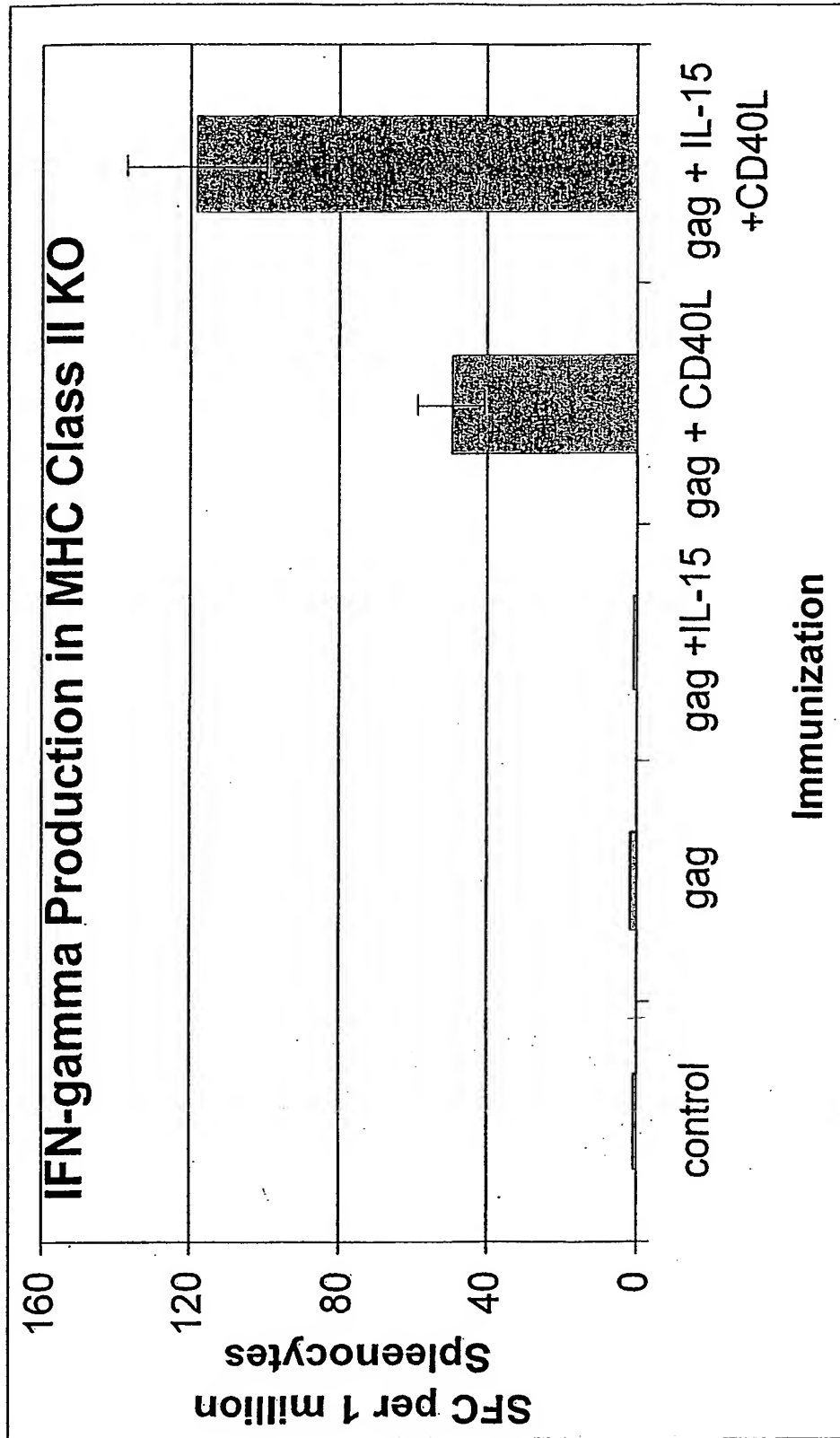


FIGURE 9

Strategy for Increasing Expression of IL-15 through Optimization of IL-15 DNA Constructs for Immunization

- ❖ Primers are designed to amplify IL-15 from start of signal peptide, thus upstream inhibitory AUGs are not present in the final IL-15 message.
- ❖ Primers are designed to include a strong KOZAK context (GCCGCCACC).
- ❖ Removal of the C-terminus negative regulatory element using PCR antisense primer design

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15 (LSP)	sense (SEQ ID NO:4)	GCGCCGCTCGAC GCCGCCACC ATGAGAA TTCGAAACCACATTGAG
	antisense (SEQ ID NO:5)	ATCGGGCTCGAG TCAAGAAGTGTGATGAACATTGG
Macaque IL-15 (LSP)	sense (SEQ ID NO:4)	GCGCCGCTCGAC GCCGCCACC ATGAGAA TTCGAAACCACATTGAG
	antisense (SEQ ID NO:5)	ATCGGGCTCGAG TCAAGAAGTGTGATGAACATTGG
Human IL-15 (SSP)	sense (SEQ ID NO:6)	GCGCCGCTCGAG GCCGCCACC ATGAGAA TTCGAAACCACATTGAG
	antisense (SEQ ID NO:7)	ATCGGGGGATCC TCAAGAAGTGTGATGAACAT
Legend: Restriction Site, KOZAK, START, STOP CODON		

FIGURE 10

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Strategy for Increasing Expression of IL-15 through Replacement of 48 amino acid Signal Peptide (LSP) with IgE leader

- ❖ Sense primers are designed to start after 48 aa ISP while antisense primer amplifies from stop site.
- ❖ Primers are designed to include a strong KOZAK context (GCCGCCACC).
- ❖ Sense primer is designed to contain the sequence for IgE leader sequence plus a ATG start site.

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15-IgE	sense (SEQ ID NO: 8)	GCCCGCGAATTC GCCGCCACC AIGGATTGGACTTGGATCTTATTTT
	(SEQ ID NO: 9)	AGTTGCTGCTGCTACTAGAGTTCAITCTAACTGGGTGAATGTAATAAGT
	antisense (SEQ ID NO: 5)	ATCGGGCTCGAG TGAAGAAGTGTGATGAACATTGG
	sense (SEQ ID NO: 8)	GCCCGCGAATTC GCCGCCACC AIGGATTGGACTTGGATCTTATTTT
Macaque IL-15-IgE	(SEQ ID NO: 9)	AGTTGCTGCTGCTACTAGAGTTCAITCTAACTGGGTGAATGTAATAAGT
	antisense (SEQ ID NO: 5)	ATCGGGCTCGAG TGAAGAAGTGTGATGAACATTGG
	sense (SEQ ID NO: 8)	GCCCGCGAATTC GCCGCCACC AIGGATTGGACTTGGATCTTATTTT
	(SEQ ID NO: 9)	AGTTGCTGCTGCTACTAGAGTTCAITCTAACTGGGTGAATGTAATAAGT

Legend: Restriction Site, KOZAK, START, STOP CODON

FIGURE 11

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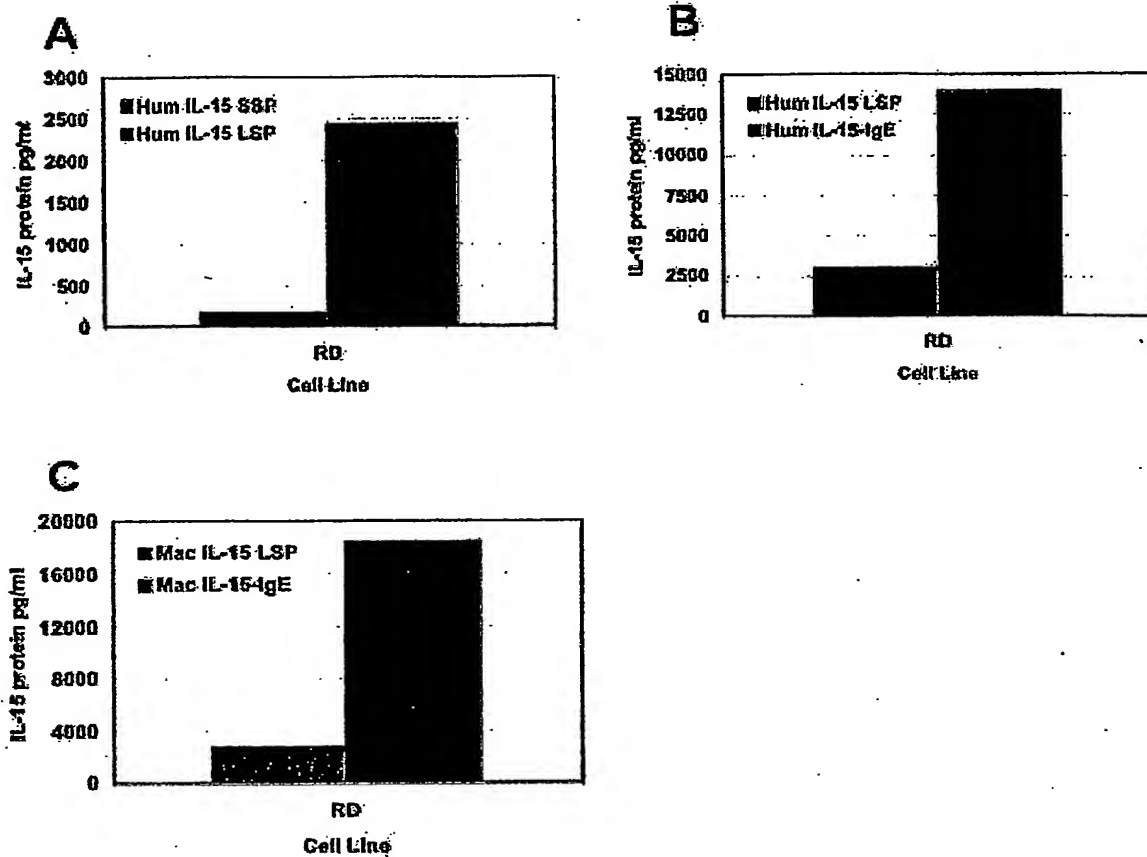


FIGURE 12

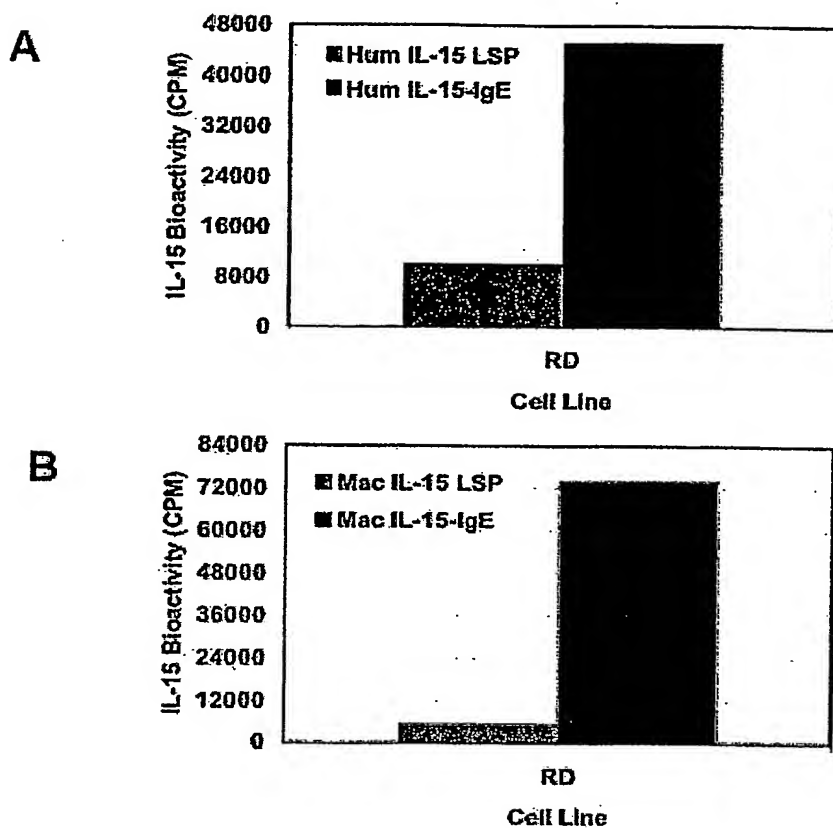


FIGURE 13

Immunization Schedule

Immunization Groups:

Naïve

Vector Control

HIV-1 Gag

HIV-1 Gag/ IL15 constructs



*Combinations of 100 µg IL15 Constructs, 50 µg GAG,
Each injection, intramuscular*

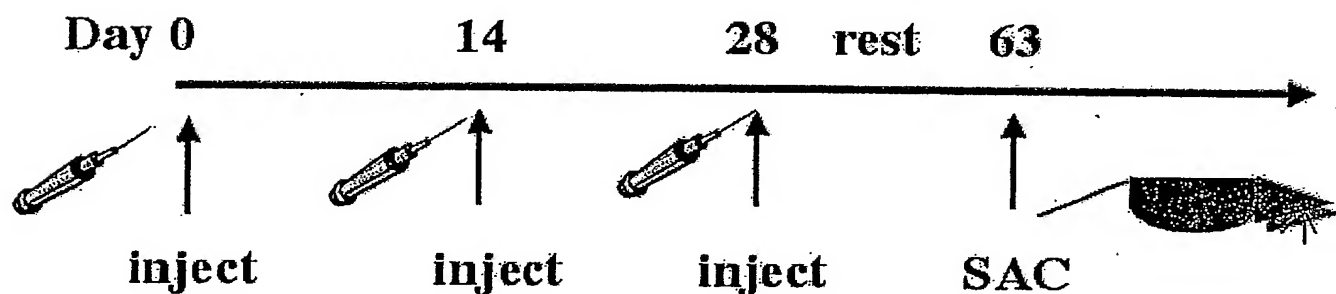


FIGURE 14

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Restimulation of antigen-specific IFN- γ production 5 Weeks Following
the 3rd immunization of HIV-1 Gag in Balb/C mice
Effect of IL-15 Constructs

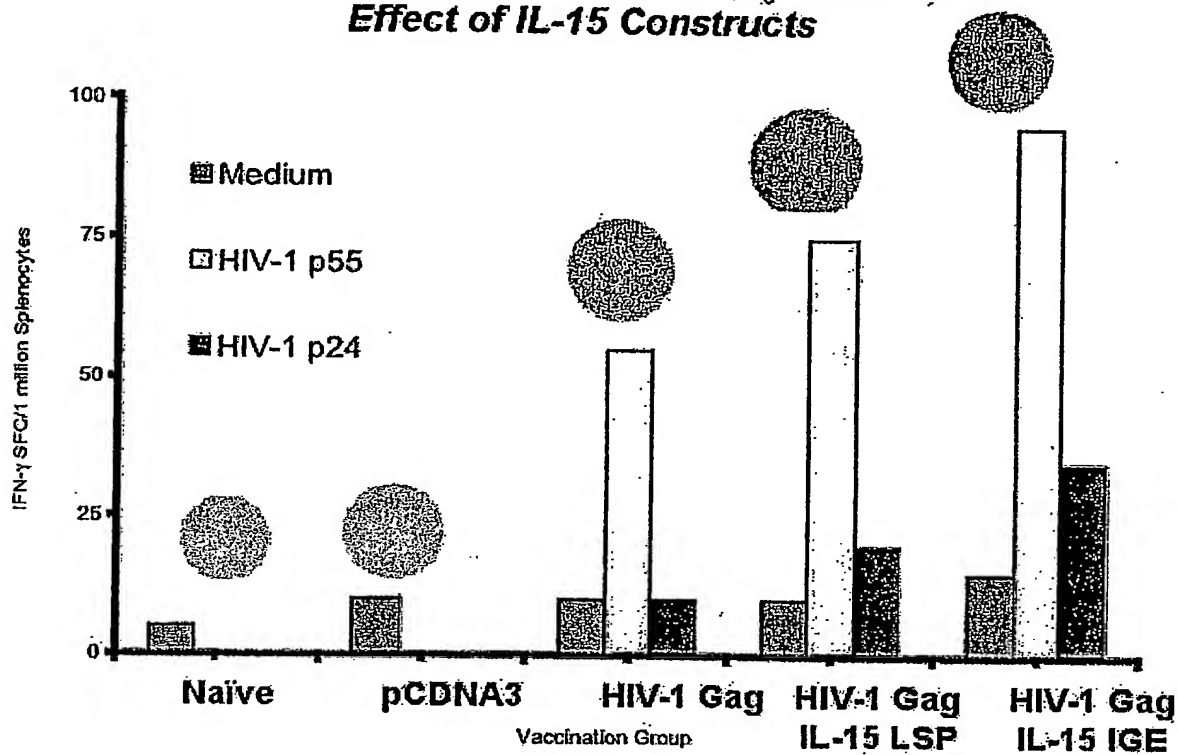
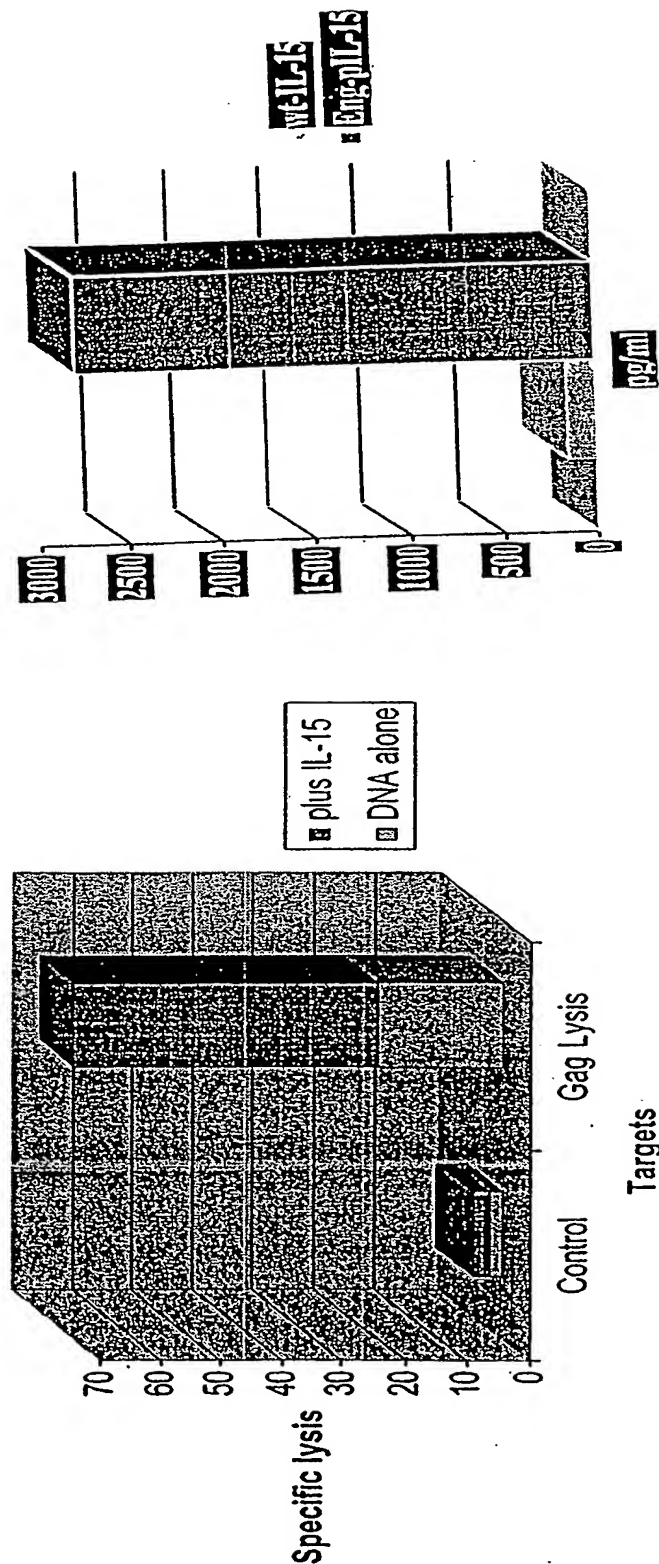


FIGURE 15

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An Engineered IL-15 Plasmid Vaccine (Kozak, AUG's removed, UTR's removed & other-30- 50X better expression)

Enhances CTL response in Vivo
Mice were immunized with HIV-1 gag expressing DNA



Grabstein et al. (1994) Science 264:965-968, Bamford et al., 1996) PNAS 93:2897-2902
Bamford et al., (1998) J. Immunol 160:4418-4426, Kozak et al., (1991) J. Cell Biol. 115:887-903

FIGURE 16

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